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MINIMAL RESIDUAL DISEASE (MRD) AND NEUROBLASTOMA TUMOR INITIATING CELLS (NTICs) IN HEMATOPOIETIC PERIPHERAL STEM CELL (HPBSC) COLLECTIONS FROM HIGH RISK NEUROBLASTOMA CHILDREN WAS ASSESSED BY GENE EXPRESSION OF *TH,MYCN* USING QUANTITATIVE REAL TIME PCR (QRT-PCR), IMMUNOPHENOTYPE ANALYSIS AND LONG TERM CULTURE INITIATING CELL (LTC-IC)

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The presence of MRD/NTICs in autologous HPBSC collections and the high frequency of disease recurrence have great concerns for transplantation. Several studies have shown that the infusion of tumor cells with the HPBSC graft is strongly associated with relapse. In this study we confirm the presence of MRD/NTICs in HPBSC collected from patients who underwent autologous transplantation.

Eighteen samples from HPBSC collected from children diagnosed with high-risk neuroblastoma by apheresis (COBE spectra) were deemed to be suitable for transplantation (lack expression of Tyrosine Hydroxylase (*TH*) and ICC negative). At the time of collection, patients were assessed to be in clinical remission (CR) or very good partial remission (VGPR). An aliquot from each collection was assessed by various methods: gene expression of *TH* and *MYCN* by qRT-PCR, surface marker immunophenotype using CD56 (NCAM), GD2 and CD9 by flow cytometry and placed on LTC-IC, after CD133 positive selection, viability and clonogenicity of cells as well as gene expression (*TH, MYCN*) were assessed at 8 weeks in culture. Undetectable gene expression (*TH or MYCN*) was observed on day 0 compared to 1.8 x0 and 1.4 x-1 in negative controls vs. 2.9x6 and 9.0x2 for human neuroblastoma cell lines. At week 8 in LTCIC there was 6.5x3 fold increased in expression of *TH* and 1.6x3 fold increased in *MYCN* gene expression in patient samples. while negative controls showed no increase of gene expression and no change in gene expression in the neuroblastoma cell lines. The patient samples also formed spheres, both adherent and non-adherent that were similar morphologically and by immunofluorescence to neurospheres generated from neural stem cells. Generation of self-renewing cells post CD 133 selection show extensive cell proliferation with limited differentiation on patient samples consistent with characteristic to TICs. In contrast, negative controls progressed into quiescence or apoptosis.

We conclude that HPBSC who were assessed as free of tumor at the time of collection show the presence of MRD/TIC detected at week 8 in LTCIC. Further studies to determine if these cells have the ability to initiate tumors need to be performed.

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ALTERNATIVE DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHILDREN WITH ACQUIRED SEVERE APLASTIC ANEMIA

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Background: Hematopoietic stem cell transplantation (HSCT) from matched sibling donor (MSD) is a standard first-line treatment for children with severe aplastic anemia (SAA). However, the management of SAA or very severe aplastic anemia (vSAA) lacking a suitable donor remains a great challenge. For those children, HSCT using unrelated donor or mismatched related donor could be a therapeutic alternative. The purpose of this study is to evaluate the outcome in children with SAA who received HSCT from donors other than matched sibling.

Patients and Method: Between July 1998 and September 2011, 24 patients received HSCT from alternative donor (AD) at Asan Medical Center Children's Hospital. We reviewed their medical records and analyzed their transplant-related parameters and outcome.

Results: Of a total of 24 patients, 15 were male and the median age at HSCT was 12.9 years, ranging from 3.0 to 21.7 years. Of 24, 9 had vSAA and 2 developed SAA after liver transplantation. Seventeen patients had received immunosuppressive treatment with antithymoglobulin before HSCT, while 7 had not. The median time from diagnosis to HSCT was 13 months, ranging from 3 to 140 months. Donors included unrelated bone marrow (U-BM) in 3, unrelated peripheral blood (U-PB) in 8, unrelated cord blood (U-CB) in 2, related haploidentical PB (H-PB) in 8 and mismatched related donor in 3 (1 BM, 2 PB). Of 24 patients, 23 (95.8%) achieved neutrophil engraftment at a median of 12 days post-HSCT. One patient (U-BM) who failed to engraft was dead despite second HSCT. Of 23 with engraftment, 2 patients (1 U-CB, 1 H-PB) died of TRM on days +157 and +81 respectively. With a median follow up of 39.8 months, The Kaplan-Meier estimated overall survival at 3 years was 86.0%.

Conclusion: In children with SAA, HSCT from AD including haploidentical family donor could be considered as a treatment option if the patients have no MSD. Given the limitation of this study such as small number of patients and short follow-up period, further trial will be necessary.

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EPSTEIN-BARR VIRUS ASSOCIATED COMPLICATIONS AND THEIR ASSOCIATION WITH ANTITHYMOCYTE GLOBULIN IN PEDIATRIC ALLOGENEIC STEM-CELL TRANSPLANTATION

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Introduction: Epstein-Barr virus (EBV) is a major concern in the context of allogeneic hematopoietic stem-cell transplantation (HSCT). EBV viremia, infection and post-transplant lympho-proliferative disorder (PTLD) are serious complications of HSCT. Those complications have been mainly associated with the use of antithymocyte globulin (ATG) and cord blood transplantation. The aim of this study was to assess the incidence of EBV viremia and PTLD in pediatric patients with and without the use ATG in their conditioning regimen.

Methods: Two-hundred and seventy-three consecutive pediatric patients who underwent 290 allogeneic HSCT at The Hospital for Sick Children, Toronto, Canada, between 2005 and 2010 were included in this study. Median age at the time of transplant was 8.5 years (range, 0.2-18.0). EBV polymerase chain reaction testing was performed weekly for the first 100 days after HSCT. A logistic regression model was constructed to assess the association between EBV viremia and the following parameters: age at time of transplant (in years), gender, use of ATG, graft versus host disease (measured as acute (grade 2 -4) and chronic), indication for HSCT (malignant vs. non-malignant), PTLD, donor EBV sero-status and stem-cell source.

Results: Of the 290 transplants performed, 127 (43.8%) used ATG. Only 8 children experienced PTLD (2.7%), all of which also experienced EBV viremia. Given this, PTLD was not included in the models. In a uni-variable analysis the use of ATG was not found to be associated with an increased risk of EBV viremia ($p = 0.88$); donor EBV sero-positivity was found to be associated with an increased risk for EBV viremia ($p<0.001$); Cord blood transplantation was associated with reduced risk for EBV viremia and PTLD ($p<0.001$).In the multi- variable analysis the use of ATG was not significantly associated with EBV viremia (OR = 0.62, 95%CI:0.30-1.28) independent of the other variables in the model. Transplantation for malignant disease and donor seropositivity status were significantly associated with EBV viremia independent of the other variables in the model (OR = 2.13, 95%CI: 1.03-4.42 and OR = 4.50, 95%CI: 2.39-8.45, respectively).

Conclusions: EBV donor seropositivity and transplantation for malignant disease is strongly associated with the development of EBV viremia. EBV-viremia is very strongly associated with the development of PTLD but the use of ATG is **not** associated with EBV viremia in our experience.